SAT0016

DEVELOPMENT OF FIBROBLAST-LIKE SYNOVIOCYTE ASSAYS FOR TARGET DISCOVERY IN RHEUMATOID ARTHRITIS

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Background: The rheumatoid arthritis (RA) synovium is characterized by an overabundance of fibroblast-like synoviocytes (FLS), which play a central role in the initiation and perpetuation of disease via multiple mechanisms. 2 FLS promote disease progression by producing high levels of proinflammatory factors, mediating angiogenesis and invading cartilage and bone, and promoting self-proliferation and resistance to apoptosis. Our current understanding of the molecular mechanisms that govern FLS-mediated inflammation in the synovial joint remains incomplete. Importantly, almost 30% of treatment-naïve early RA patients exhibit a strong fibroblast-like synoviocyte (FLS) phenotype that correlates with relatively poor response to disease-modifying antirheumatic drugs. 3 Importantly, almost 30% of treatment-naïve early RA patients exhibit a strong fibroblast-like synoviocyte (FLS) phenotype that correlates with relatively poor response to disease-modifying antirheumatic drugs. 3

Methods: Two groups of C57BL/6J mice were used, in the control group, mice were treated with physiological serum and in the experimental group with a ROCK inhibitor (Y-27632). Arthritis was induced by intraperitoneal injection of 100 µl of K/BxN serum on days 0 and 2. In the experimental group, mice were treated with intraperitoneal injections of 10 mg/kg from day 0 until sacrifice, on day 10. Control mice were treated with the same volume of physiological serum. Arthritis was assessed by two observers using a semiquantitative clinical score. For histological analysis, it was decided to obtain the right ankle joints and foot. Tissues were fixed in formalin for 6h and were decalcified and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and toluidine. Finally, total RNA was obtained from wrist and ankle joints of mice and the expression of inflammatory mediators and metalloproteinases was analyzed by real-time PCR.

Results: Arthritis was induced in C57BL/6J mice, which were treated with Y-27632 (ROCK inhibitor) or with physiological serum. The incidence of arthritis was 100% in both groups of mice and there were no differences in the course of the disease. Clinical score was significantly lower in the Y-27632-treated mice, all along the follow-up, compared with controls. Similar results were observed in the histological analysis. We also analyzed the effect of ROCK inhibitor on the inflammatory response of K/BxN serum-transfer induced arthritis. This analysis revealed that expression of IL6, IL1β, CXCL1, MMP3, MMP9 and MMP13 were significantly decreased in Y-27632-treated mice compared with control mice. In addition, TNF and NOS2 expression was reduced in Y-27632-treated mice to reach the same levels that observed in C57BL/6J mice without arthritis.

Conclusion: These results indicate that the inhibition of the Rhino-ROCK pathway decreases the severity of arthritis in the K/BxN serum transfer model, and point to ROCK as potential therapeutic target for RA. Supported: ISCIII / P117 / 01660 / RETICS Program, RD16 / 00120014 / Cofinanced FEDER.

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SAT0017

METABOLIC CHANGES INDUCED BY ANTI-MALONDIALDEHYDE/MALINDIALDEHYDE-ACETALDEHYDE ANTIBODIES PROMOTE OSTEOCLAST DEVELOPMENT

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Background: Malondialdehyde (MDA) is a highly reactive compound produced by lipid-peroxidation in situations associated with oxidative stress. MDA can irreversibly modify proteins residues such as lysine, arginine and histidine. In addition, MDA adducts can further react with acetaldehyde to generate malonaldehyde-acetaldehyde (MAA) modifications. Such modifications can give rise to immunogenic neo-epitopes that are recognized by autoantibodies. In fact, anti-MDA/MAA IgG antibodies are significantly increased in the serum of patients with autoimmune diseases, such as rheumatoid arthritis (RA) (1) and systemic lupus erythematosus (2). Recently, we have shown that anti-MDA/MAA IgG antibodies are able to promote osteoclast (OC) differentiation in vitro (1).

Methods: To examine the ability of small-molecule inhibitors to block the production of interleukin (IL)-6 and matrix metalloproteinase (MMP)-3 in response to stimuli. To create a physiologically relevant stimulus, a surrogate synovial fluid cocktail (composed of 12 factors) was defined and titrated for optimal concentration selection. Small-molecule inhibitors (N=170) of diverse biological pathways were screened using the full cocktail or individual stimulation (TNFα, IL-1α, or IL-17) to characterize assay performance. In addition, an FLS platelet-derived growth factor (PDGF)-mediated migration screening assay was developed using a live cell imaging system (Incucyte) to quantify real-time FLS migration.

Results: Due to the variability and limited volume of synovial fluid, we developed a surrogate synovial fluid cocktail to mimic the relevant stimulation of RA-FLS in the inflamed joint. The surrogate cocktail was composed of 12 factors: TNFα, IL-1α, IL-17, IFNγ, OSM, LIF, GM-CSF, IP-10, VEGF, PDGF, AREG, and FGF2. Individual titration of these factors demonstrated that only 3 stimulatory factors (TNFα, IL-1α, and IL-17) resulted in a robust increase of IL-6 production. Importantly, when all 12 factors were combined, a synergistic increase in IL-6 and MMP-3 production by FLS was observed. Screening results identified several reference compounds, including an inhibitor of transforming growth factor-beta-activated kinase 1 (TAK1), that was previously reported to block cytokine secretion in FLS. Treatment with this compound showed complete inhibition of IL-6 and MMP-3 secretion. In addition to the cytokine secretion assay, treatment of FLS with this TAK1 inhibitor resulted in almost complete inhibition of migration (Fig. 1).

Conclusion: Novel FLS assays were developed to discover new targets and interrogate pathways involved in multiple disease-driving mechanisms of FLS in RA.

References:

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SAT0018

ACETALDEHYDE ANTIBODIES PROMOTE OSTEOPOROSIS AND MALONIALDEHYDE ACETALDEHYDE ANTIBODIES PROMOTE OSTEOCLAST DEVELOPMENT

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Background: Fibroblast-like synoviocytes (FLS) are pivotal in inflammation and joint damage of rheumatoid arthritis (RA). These cells acquire an aggressive and invasive phenotype and secrete inflammatory mediators, metalloproteinases and cathepsins that perpetuate inflammation and lead to cartilage and bone damage. We have previously shown that non-canonical Wnt5a pathway is involved in the aggressive phenotype of FLS by increasing their migration and invasion ability, and by stimulating the inflammatory response. The non-canonical Wnt signaling pathway included the planar cell polarity (PCP), with the activation of Rho and Rac GTPases, and the Wnt/Ca2+ pathways. We have also shown that Wnt5a contributes to the aggressive phenotype of RA FLS by binding to RYK receptor, through Rho-ROCK pathway and the activation of MAPKs, ERK and p38, as well as the activation of AKT and GSK3β.

Objectives: To elucidate the therapeutic potential of the ROCK inhibitor (Y-27632) in the K/BxN serum transfer arthritis model.

Methods: Two groups of C57BL/6J mice were used, in the control group, mice were treated with physiological serum and in the experimental group with a ROCK inhibitor (Y-27632). Arthritis was induced by intraperitoneal injection of 100 µl of K/BxN serum on days 0 and 2. In the experimental group, mice were treated with intraperitoneal injections of 10 mg/kg from day 0 until sacrifice, on day 10. Control mice were treated with the same volume of physiological serum. Arthritis was assessed by two observers using a semiquantitative clinical score. For histological analysis, it was decided to obtain the right ankle joints and foot. Tissues were fixed in formalin for 6h and were decalcified and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and toluidine. Finally, total RNA was obtained from wrist and ankle joints of mice and the expression of inflammatory mediators and metalloproteinases was analyzed by real-time PCR.

Results: Arthritis was induced in C57BL/6J mice, which were treated with Y-27632 (ROCK inhibitor) or with physiological serum. The incidence of arthritis was 100% in both groups of mice and there were no differences in the course of the disease. Clinical score was significantly lower in the Y-27632-treated mice, all along the follow-up, compared with controls. Similar results were observed in the histological analysis. We also analyzed the effect of ROCK inhibitor on the inflammatory response of K/BxN serum-transfer induced arthritis. This analysis revealed that expression of IL6, IL1β, CXCL1, MMP3, MMP9 and MMP13 were significantly decreased in Y-27632-treated mice compared with control mice. In addition, TNF and NOS2 expression was reduced in Y-27632-treated mice to reach the same levels that observed in C57BL/6J mice without arthritis.

Conclusion: These results indicate that the inhibition of the Rhino-ROCK pathway decreases the severity of arthritis in the K/BxN serum transfer model, and point to ROCK as potential therapeutic target for RA. Supported: ISCIII / P117 / 01660 / RETICS Program, RD16 / 00120014 / Cofinanced FEDER.

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**Results:** In CIA in mice all sham animals developed arthritis, compared to only 14% following six times per day SNS (p < 0.001) in a prophylactic setting. In contrast, 85% of the animals developed arthritis (p = 0.36) when SNS was applied only once a day. In both groups the development of OCs was monitored by light microscopy following tartrate-resistant acid phosphatase (TRAP) staining and erosion area on synthetic calcium phosphate-coated plates. Three different recombinant human monoclonal anti-MDA/MAA antibodies, cloned from single synovial B cells of RA patients, control antibodies and Fab fragments of the antibodies were added to OC cultures. Glycolysis was inhibited by 2-deoxyglucose, and Fc-gamma receptor I or II by anti-CD64 or anti-CD16 neutralizing antibodies. IL-8 levels were measured by enzyme linked immunosorbent assay. Cellular metabolism was monitored using Seahorse XF Analyzer (extracellular acidification rate and oxygen consumption) and a colorimetric L-Lactate assay.

**Conclusion:** These studies demonstrate that SNS suppresses pro-inflammatory cytokine production, and reduces clinical symptoms in mice with CIA which is at least partially mediated by the β-AR. The additive effect of anti-TNF in reducing the clinical scores demonstrates that that mechanism of action of SNS is not primarily mediated by reducing TNF levels. Moreover, anti-TNF potentiating the inhibitory effect of SNS is supporting a combined use of these treatments, or even a combination of SNS with other biologicals, to treat RA, potentially getting more patients closer to remission. In conclusion, the data is providing compelling scientific rationale and pre-clinical evidence that splenic neuromodulation might be a new treatment modality for RA.

**References:**